Genetic Characterization of *small ovaries*, a Gene Required in the Soma for the Development of the Drosophila Ovary and the Female Germline

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ABSTRACT

The *small ovary* gene (*sov*) is required for the development of the Drosophila ovary. Six EMS-induced recessive alleles have been identified. Hypomorphic alleles are female sterile and have no effect on male fertility, whereas more severe mutations result in lethality. The female-sterile alleles produce a range of mutant phenotypes that affect the differentiation of both somatic and germline tissues. These mutations generally produce small ovaries that contain few egg cysts and disorganized ovarioles, and in the most extreme case no ovarian tissue is present. The mutant egg cysts that develop have aberrant morphology, including abnormal numbers of nurse cells and patches of necrotic cells. We demonstrate that *sov* gene expression is not required in the germline for the development of functional egg cysts. This indicates that the *sov* function is somatic dependent. We present evidence using loss-of-function and constitutive forms of the somatic sex regulatory genes that *sov* activity is essential for the development of the somatic ovary regardless of the chromosomal sex of the fly. In addition, the genetic mapping of the *sov* locus is presented, including the characterization of two lethal *sov* alleles and complementation mapping with existing rearrangements.

THE developing Drosophila egg is a mosaic of somatic and germline cells whose coordinate differentiation is essential for normal oogenesis. An active interaction between the germline and soma controls the deposition of yolk protein into the egg, the production of the chorion egg "shell" (MAHOWALD and KAMBYSELLIS 1980) and dorsal-ventral patterning in the egg chamber and embryo (WIESCHAUS 1979; SCHÜPBACH 1987; STEVENS *et al.* 1990). In addition, the proliferation and differentiation of both male and female germ cells are influenced by the sexual identity of the somatic gonad. Pole cell transplantation studies demonstrate that functional gametes are only produced when *X/X* germ cells develop in ovaries and *X/Y* germ cells in testes (VAN DEUSEN 1976; MARSH and WIESCHAUS 1978; SCHÜPBACH 1982; STEINMANN-ZWICKY *et al.* 1989). Therefore, it appears that the genotype of the germline is not sufficient to support spermatogenesis or oogenesis in somatic tissue of the inappropriate sex. In fact, sex-specific somatic signals can induce germ cells to undergo a differentiation pathway that is contrary to what would be expected from their *X*:A ratio. When *X/X* germ cells were transplanted into testes, they attempted to undergo what appeared by morphological criteria to be the initial stages of spermatogenic differentiation (STEINMANN-ZWICKY *et al.* 1989). Apparently the male soma can "impose" its sexual identity on the *X/X* germline.

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The germline is in physical proximity to the somatic gonad beginning at early stages in embryogenesis. The pole cells migrate during germ band extension to the mesodermal precursors of the gonad. These somatic cells can differentiate into either ovary or testes depending on the action of the somatic sex regulatory genes. Sexual dimorphism in the gonads become visible during the early larval stages both in terms of gonad size and germ cell morphology. This sexual differentiation is dependent on sex-specific interactions between the soma and germline that occur during this period (STEINMANN-ZWICKY 1994).

Despite this close interaction between the soma and germline, the sexual differentiation of these tissues is regulated by two distinct sets of genes. Somatic sex determination depends on the interpretation of the *X*:A ratio by the *Sex-lethal* (*Sxl*) gene (reviewed in PARKHURST and MENEELY 1994). A female *X*:A ratio of 1:1 (*X/X*) activates the *Sxl* RNA splicing activity that causes the *transformer* (*tra*) gene to produce a female-specific product. The *tra* function acts with the product of another unlinked gene, *transformer-2* (*tra-2*), to control the sex-specific expression of the functionally dimorphic *doublesex* (*dsx*) gene. Mutations in any of these genes can alter the somatic sexual differentiation of the fly. For example, loss-of-function mutations in *tra* cause *X/X* flies to develop as males with fully developed male somatic structures (STURTEVANT 1945; BROWN and KING 1961). However, these sexually altered flies are sterile with only rudimentary germline development, indicating that the germline is not similarly sexually

transformed (BROWN and KING 1961; NÖTHIGER *et al.* 1989). Furthermore, pole cell transplantation experiments demonstrate that *X/X* germ cells mutant for *tra*, *tra-2* or *dsx* can develop normally if placed in a female somatic environment (MARSH and WIESCHAUS 1978; SCHÜPBACH 1985). These results suggest the existence of a separate genetic pathway to regulate sexual differention of the germline. Several genes have been implicated in this process, including *otu*, *ovo*, *Sxl* and *sans fille* (*snf*), based on morphological and molecular observations that suggest mutations in these genes can cause *X/X* germ cells to take on some male characteristics. However, this presumed sexual transformation is incomplete and subject to other interpretations (BAE *et al.* 1994).

Later in development, the differentiation of the egg cysts becomes dependent on interactions that occur between the germline and the somatically derived follicle cells. By the late larval and pupal stages, the female germline has begun the process of differentiating into egg chambers. Initially, germline stem cells divide asymmetrically to produce a daughter stem cell and a cystoblast. The cystoblast undergoes four mitotic divisions, each characterized by incomplete cytokinesis, to form a 16-cell syncytium. One of these cells becomes the oocyte, whereas the other 15 differentiate into nurse cells that provide much of the material for the maturation of the oocyte. As the 16-cell syncytium develops, it becomes surrounded by follicle cells and together these form the egg chamber.

Follicle cells have specialized behaviors and functions that are essential for the development of the egg. For example, the delamination of follicle cells at the anterior end of the cyst and their subsequent differentiation into stalk cells act to separate individual egg chambers as they leave the germaria (KING 1970). Polar follicle cells that become localized at the anterior-posterior ends of the egg chamber will eventually give rise to dorsal appendages and may also be required for anterior-posterior microtubule organization (RUOHOLA *et al.* 1991; CLARK *et al.* 1994). Border follicle cells migrate into the lumen of the developing egg chamber and act to separate the growing oocyte from the nurse cells. These follicle cells are also required for the development of the micropyle (MONTELL *et al.* 1992). Mutations that disrupt the differentiation of specific follicle cells can have dramatic effects on the morphology of the ovary and the viability of the female germline. For example, the neurogenic *Notch* and *Delta* genes are also required in the ovaries for the establishment of follicle cell fate and oocyte polarity. Mutations in these genes result in fused and disorganized egg chambers and are often associated with necrotic germ cells (RUOHOLA *et al.* 1991).

These results indicate that the development of the female germline is dependent on the sexual state of the soma, as controlled by the somatic sex determination genes, as well as on the genes that control the differentiation of the follicle cells. How the female germline is affected by these somatic factors is not well understood. In this manuscript we examined the soma–germline interaction by characterizing a gene, *small ovaries* (*sov*), that is required both for the formation of the somatic ovary and for the development of the female germline. We demonstrate that the expression of the *sov* gene is required in the soma for both the development of the somatic ovary and the normal differentiation of the female germline. This ovary-specific *sov* function is dependent on regulation by the somatic sex determination genes. We suggest that *sov* serves to mediate at least a subset of the interactions that occur between the somatic and germline tissues.

MATERIALS AND METHODS

Fly strains: The sov^{1-3} alleles were isolate in an EMS mutagenic screen designed to identify sex-specific sterility (MOHLER 1977). Descriptions of other mutations and balancer chromosomes used in this study are found in LINDSLEY and ZIMM (1992). Flies were raised on a standard cornmeal, molasses, yeast, agar media containing propionic acid as a mold inhibitor and supplemented with live yeast.

EMS mutagenesis used to isolate lethal *sov* alleles: sov^{ML150} and sov^{ML185} were isolated in an F2 screen for EMS-induced *X*-linked lethal and sterile mutations. W^{1118}/Y males were fed 25 mM EMS in 5% sucrose for 24 hr using standard protocols (ASHBURNER 1989). The mutagenized (w^{1118*}/Y) males were mated enmasse to *FMO/ClB* females and the w^{1118*}/FMO and W^{1118*}/ClB female progeny were individually mated to *FMO/Y* males. The progeny from each pair mating was examined for the presence of an *X*-linked lethal by the absence of B^+ (W^{1118*}/Y) males. Pair matings with viable mutagenized *X* chromosomes were tested for the presence of female-sterile lesions. In these cases the mutagenized chromosomes were made homozygous and the resulting females were tested for fertility. From 1821 mutagenized chromosomes we obtained 756 (41.5%) *X*-linked lethals and 66 (3.6%) *X*-linked female steriles. We tested each mutation against sov^2 for female fertility. Two of the lethal lines were shown to be allelic to *sov*.

Morphological analyses of gonads: The morphology of the mutant gonads were examined by either Feulgen or DAPI staining, both of which specifically label nuclei. Fly cultures were kept under uncrowded conditions at 25°. Female flies of the appropriate genotypes were aged 2–3 days after eclosion at 25°. The ovaries were dissected in phosphate-buffered saline (PBS; 130 mM NaCl, 7 mM $Na_2HPO_4.2H_2O$, 3 mM $NaH_2PO_4.2H_2O$) and then stained by Feulgen reaction using a modification of the procedure described in GALIGHER and KOZLOFF (1971). Ovaries were hand dissected and fixed in Carnoy's solution (1:4 acetic acid:ethanol) for 2–3 min. After fixation, the ovaries were incubated in 5 N HCl for 3–4 min. This was followed by incubation in Feulgen reagent until the nuclei were appropriately stained. Staining was stopped by a 5-min incubation in dilute sulfuric acid. The ovaries were dehydrated by a series of washes in 10%, 30%, 50%, 70%, 90%, 100% ethanol. The stained ovaries were cleared in xylene and mounted in permount. Specimens can be visualized under visible light or fluorescence using a green excitation filter.

For DAPI staining, adult gonads were dissected in PBS and

then incubated in 50% fixative:50% heptane in a covered depression slide with agitation from a rotary shaker for 3 min. The tissue was rinsed three times in in PBS + 0.1% Triton and incubated in DAPI solution (0.5 μg/ml in 180 mM Tris-HCl, pH 7.5) for 1 hr to fluorescently stain nuclei. The preparation was washed for 20 min five times with PBS. The tissue was mounted in 50% glycerol in PBS. The stock solutions used for this procedure were as follows: solution B, 1.4 g/l Na_2HPO_4, 0.1 g/l KH_2PO_4, take to pH 7 with NaOH, and solution C, 6.75 g/l NaCl, 6.63 g/l KCl, 0.66 g/l $MgSO_4.7H_2O$, 0.54 g/l $MgCl_2.6H_2O$, 0.33 g/l $CaCl_2.2H_2O$ in 3.7% formaldehyde. The fixative was made up by mixing nine parts solution C with 10 parts solution B. DAPI-stained specimens were observed under fluorescence using a UV excitation filter.

Germline clonal analysis: Germline clones were produced by the well-established dominant female-sterile procedure (PERRIMON and GANS 1983). The dominant female-sterile allele, ovo^{D1} (or *Fs(1)K1237*), blocks oogenesis when present in one copy in the germline stem cells. The progeny from the mating of *y cv sov⁻ v f/FM6* females to ovo^{D1} v^{24}/*Y* males were irradiated with 1000 rads from a ^{137}Cs gamma source at 44–52 hr postoviposition to induce mitotic recombination in the germline. Clones induced by this method often occupy several ovarioles (WIESCHAUS and SZBAD 1979). Irradiated females of the genotype *y* ovo^+ *cv* sov^- *v f/*ovo^{D1} sov^+ v^{24} were tested for fertility by matings with *y cv v f/Y* males. Clones resulting from recombination events proximal to *f* (and therefore *sov* and *ovo* as well) must be ovo^+ sov^-, producing eggs of the genotype *y* ovo^+ *cv* sov^- *v f*. These proximal clones were identified by the production of progeny that were yellow, crossveinless, vermillion and forked when crossed to *y cv v f/Y* males. Confirmation that the recombinant chromosomes were sov^- came by complementation testing against sov^2.

RESULTS

***sov* mutations affect both somatic ovary and germline development:** A normally developing ovary consists of egg cysts organized in linear arrays called ovarioles. Egg chambers of different developmental stages are found in each ovariole (ovl), with the least mature cysts located apically and the mature yolky oocytes (y) arranged near the oviduct (Figure 1A). *sov* mutations affect both the somatic and germline components of the ovary, affecting the organization of the gonad as well as the differentiation and viability of the germ cells. Three EMS-induced female-sterile *sov* alleles had been previously identified (MOHLER and CARROLL 1984) and were used in the characterization of the *sov* mutant phenotype. Because the *sov* mutations result in a range of ovarian phenotypes, we could not unambiguously determine the relative severity of the different alleles, although mutant combinations with sov^3 generally produce the most severely affected ovaries (Table 1).

The most common mutant phenotype obtained is the disruption of ovariole structure that result in ovaries (ov) containing a haphazard arrangement of the cysts (Figure 1B; Table 1). In many cases, ovarioles do not appear to form at all, producing large sacs filled with irregularly shaped cysts containing a mix of yolk globules, cells with large nuclei that resemble nurse cells and cells with pycnotic nuclei that appear necrotic (Figure 1C). The mutant ovaries are often associated with unencysted polyploid cells located in the oviduct. These are morphologically similar to nurse cells, perhaps resulting from aberrant cyst formation or the degeneration of follicle cells. In the most severely affected ovaries, the oviducts (ovd) end in small nubs (nb) that are absent germ cells and somatic ovarian tissue (Figure 1D). These could result from necrosis, reduced proliferation of the ovarian tissue or from a failure of the oviduct to join with the female gonad during development. In most cases, we believe that the nub phenotype occurs when the ovary lobe fails to develop, because we usually cannot find a detached gonad elsewhere in the abdomen. Occasionally, however, a failure of the gonad and oviduct to attach must occur as free-floating clusters of egg chambers are sometimes found.

In addition to these affects on the somatic structure of the ovary, *sov* mutations also affect the viability, proliferation and differentiation of the germline. *sov* mutant ovaries often contain egg cysts that contain fewer than the normal 15 nurse cells. These could result from cell death as the cysts often contain condensed, irregularly shaped nuclei that are associated with degenerating nurse cells (KING 1970). However, some hyponumerary cysts appear to be undergoing advanced stages of oogenesis without evidence of necrosis (Figure 1E). This suggests that the *sov* mutations can affect the number of mitotic divisions that individual cystoblasts undergo, without affecting their capacity to differentiate in a female-specific manner. Hypernumerary cysts are also found that can contain more than the normal 15 nurse cells (Figure 1F). To test whether these cysts resulted from the fusion of egg chambers or increased germ cell proliferation, we examined the number of ring canals that form in the hypernumerary cysts. In normal oogenesis the cystoblast undergoes four mitotic rounds associated with incomplete cytokinesis to produce a 16 cystocyte syncytium. The cytoplasmic bridges connecting the cystocytes are associated with a ring canal that can be visualized using phalloidin, an actin-specific fluorescent stain. Therefore, the number of ring canals indicates the number of cell divisions that each cystocyte has undergone, with a maximum of four divisions in normal oogenesis. In *sov* mutant cysts, we found no germ cells that contained more than four ring canals, and most had only one (data not shown). We therefore believe that most hypernumerary cysts occur by the fusion of one or more egg chambers containing cysts undergoing the normal number of mitotic divisions. However, we cannot preclude the possibility of some aberrant proliferation, particularly if it occurs with complete cytokinesis and no ring canal formation.

sov mutations can also result in abnormal germ cell differentiation. Cysts often form that are phenotypically similar to the ovarian tumor egg chambers (tc) that

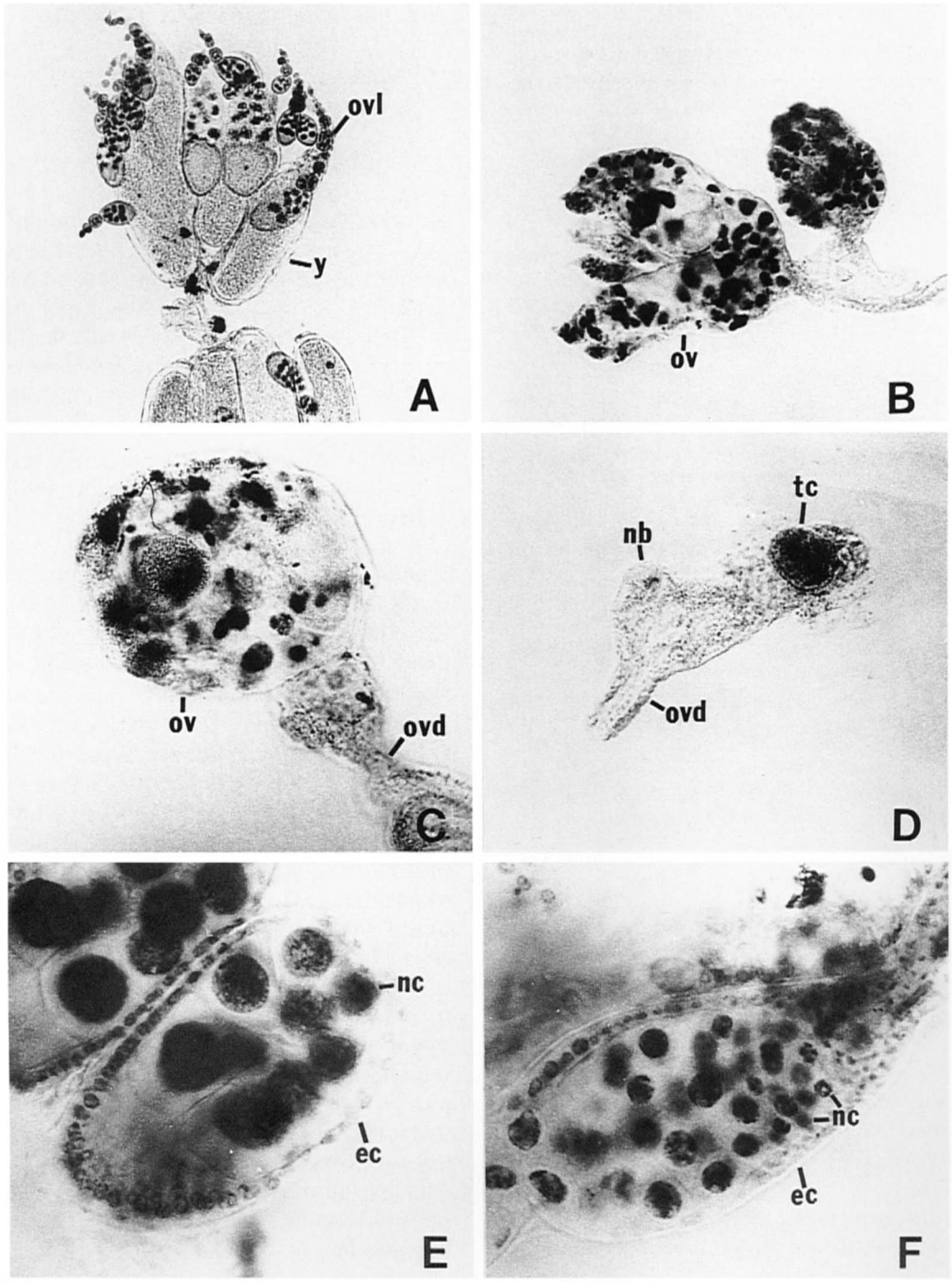

FIGURE 1.—The morphologies of wild-type and *sov* mutant ovaries. A wild-type ovary (A) is compared with the range of mutant ovarian phenotypes that result from the genotype sov^{4} *l(1)EA42/y cv* sov^{2} (B–F). The mutant phenotypes are similar to those obtained from other *sov* allele combinations. Ovaries are stained with the nucleic acid-specific Feulgen reagent and photographed under bright field optics. Nuclei stain dark. B, C and E were photographed under twofold higher magnification than A, D and F. (A) Wild-type ovary with ovarioles and yolky egg cysts. (B) Mutant ovary with some ovariole structure and aberrant egg chambers. (C) Disorganized ovary with no ovarioles. (D) Oviduct with one small lobe containing a tumorous egg cyst and a "nub." (E) Mutant cyst containing eight nurse cells (normally have 15 nurse cells). (F) Mutant cyst containing ≥30 nurse cells. ec, egg chamber; H, hypernumerary cysts; h, hyponumerary nurse cell cysts; nb, nub; nc, nurse cell; ovd, oviduct; ovl, ovariole; tc, tumorous cyst; y, yolky egg chamber.

TABLE 1

Comparison of *sov* mutant ovarian phenotypes

Genotype	Ovary lobes with ovarioles[a]	Disorganized ovary, no ovarioles[b]	No ovary lobes (nubs)[c]
$\frac{+}{+}$	1.00 (20/20)	0.00 (0/20)	0.00 (0/20)
$\frac{sov^1}{sov^1}$	0.19 (5/26)	0.54 (14/26)	0.27 (7/26)
$\frac{sov^2}{sov^2}$	0.40 (34/84)	0.49 (41/84)	0.11 (9/84)
$\frac{sov^3}{sov^3}$	0.02 (2/92)	0.28 (26/92)	0.70 (64/92)
$\left(\frac{EA42}{EA42}\right)^d$	0.14 (9/66)	0.42 (28/66)	0.44 (29/66)
$\frac{sov^1}{sov^2}$	0.52 (28/54)	0.29 (16/54)	0.19 (10/54)
$\frac{sov^3}{sov^1}$	0.53 (50/94)	0.38 (36/94)	0.09 (8/94)
$\frac{sov^3}{sov^2}$	0.12 (27/223)	0.38 (84/223)	0.50 (112/223)
$\frac{EA42}{sov^2}$	0.03 (2/68)	0.48 (33/58)	0.48 (33/68)
$\frac{sov^{ML150}}{sov^2}$	0.00 (0/126)	0.13 (16/126)	0.87 (110/126)
$\frac{sov^{ML185}}{sov^2}$	0.00 (0/111)	0.02 (2/111)	0.98 (109/111)
$\frac{sov^{ML150}}{EA42}$	0.00 (0/52)	0.00 (0/52)	1.00 (52/52)
$\frac{sov^{ML185}}{EA42}$	0.00 (0/20)	0.15 (3/20)	0.85 (17/20)

Parental cross: sov^x/Balancer (*FM6* or *FM0*) × sov^y/Y. Values in parentheses are number of labes per total.

[a] Ovary has ovarioles but defective egg chambers.

[b] Ovary is disorganized, contains germ cells but no discernable ovarioles.

[c] No somatic or germline gonadal tissue present, oviducts end in nubs.

[d] Because *l(1)EA42* is a recessive lethal, homozygous and hemizygous *l(1)EA42* flies were obtained in combination with the duplication $Dp(1,3)sn^{13a1}$. Parental cross: $l(1)EA42/FM6 \times l(1)EA42/Y; Dp(1,3)sn^{13a1}/TM6$. Full genotypes: sov^1 = y cv sov^1 v f; sov^2 = y cv sov^2; sov^3 = y cv sov^3 v f; *EA42* = *l(1)EA42*; $sov^{ML150} = w^{1118}sov^{ML150}$; $sov^{ML185} = w^{1118}sov^{ML185}$.

develop from some female-sterile mutations, including *Sex-lethal* and *ovarian tumor* (KING 1979; KING and RILEY 1982; PERRIMON *et al.* 1986; SALZ *et al.* 1987) (Figure 1D). The tumorous cysts are filled with hundreds to thousands of small germ cells that fail to undergo female differentiation. The mutant egg cysts also frequently contain irregularly shaped pycnotic nurselike cells that may represent abnormal development or cell death (Figure 1, B and C).

TABLE 2

Effect of *sov* mutations on male sterility

$$\frac{w}{w} \times \frac{ycv(sov^2)}{Y}$$

Genotype	% fertile males	Average no. progeny/male[a]
$\frac{ycv}{Y}$	87 (20/23)	76.8 ± 6.7
$\frac{ycv\ sov^2}{Y}$	100 (32/32)	90.4 ± 3.7

[a] Values are means ± SE.

In contrast to the ovary, testis morphology is not affected by any combination of the three *sov* alleles. sov^- males are fertile and contain mature testes that have wild-type pigmentation and coiling. No morphological aberrations in spermatogenesis can be discerned. To examine more subtle effects on spermatogenesis, males mutant for sov^2 were individually crossed to sov^+ females to test for fertility and fecundity. We found no increase in male sterility nor was the number of progeny produced by each male deleteriously affected (Table 2). Therefore, it appears the female-sterile alleles of *sov* do not have a male function.

Germline clonal analysis demonstrates that *sov* is somatic-line dependent: Mutations in the *sov* gene affect the development of both somatic and germline ovarian cells. This could reflect a requirement in both tissues for *sov* expression. Alternatively, it is possible that *sov* is expressed in only one tissue but is required in a cell nonautonomous manner in the other. To examine this question, we created mosaic females in which the somatic tissue carried a wild-type *sov* allele and the germline was homozygous mutant. If the mosaic animals remain sterile, this would indicate that the requirement for *sov* activity in female germ cells is cell autonomous. Alternatively, the production of progeny would mean that *sov* expression in the somatic tissue is sufficient to support oogenesis even in the absence of germline *sov* function.

Germline clones were generated by the dominant female-sterile technique in first instar larvae (PERRIMON and GANS 1983). Flies heterozygous for sov^- and the dominant female sterile mutation, ovo^{D1}, were irradiated to induce mitotic crossing over. In the absence of recombination, the adult females are sterile due to the presence of ovo^{D1}. Mitotic crossing over proximal to both the dominant female-sterile mutation and *sov* will produce recombinant germline cells that are homozygous for sov^- and wild-type for the *ovo* gene. If the

TABLE 3

Germline clonal analysis

Genotype of irradiated female[a]	No. irradiated females	Fertile mitotic crossovers resulting in ovo^+ germ cells[b]
I. $\frac{ovo^{D1}\ sov^+}{ovo^+\ sov^1}$	406	12[b] (sov^- ovo^+)
II. $\frac{ovo^{D1}\ sov^+}{ovo^+\ sov^3}$	142	8[b] (sov^- ovo^+)
III. $\frac{ovo^{D1}\ sov^+}{ovo^+\ sov^+}$	142	8 (sov^+ ovo^+)
IV. $\frac{ovo^{D1}\ sov^+}{ovo^+\ sov^2}$	554	16[c] (sov^- ovo^+)
V. $\frac{ovo^{D1}\ sov^+}{ovo^+\ sov^+}$	391	20[d] (sov^+ ovo^+)

[a] First instar larvae were irradiated from the crosses of I, *y cv* sov^1 *v f / FM0* × ovo^{D1} v^{24}*/Y;* II, *y cv* sov^3 *v f,/FM0* × ovo^{D1} v^{24}*/Y;* III, *y cv v f,/y cv v f* × ovo^{D1} v^{24}*/Y;* IV, *y cv* sov^2*,/FM0* × ovo^{D1} *v24/Y;* V, w^{1118}*,/FM0* × ovo^{D1} v^{24}*/Y.*

[b] Progeny derived from crossovers were f^-, indicating a crossover event proximal to ovo^{D1} and *sov* resulting in ovo^+ sov^-/ovo^+ sov^- germ cells.

[c] All progeny carried the sov^2 allele and were v^+, indicating they were derived from ovo^+ sov^-/ovo^+ sov^- germ cells.

[d] All progeny carried the w^{1118} allele, indicating they were derived from a w^{1118} $ovo^+/w^{1118}ovo^+$ germ cell.

expression of *sov* is not required in the germline, then these cells should be able to produce functional eggs. Alternatively, a cell autonomous germline requirement for *sov* activity would preclude the induction of fertile sov^- clones. As shown in Table 3, fertile clones were induced with the sov^1, sov^2 and sov^3 alleles, indicating that *sov* function is not required in the female germline for fertility despite the severe effects of *sov* mutations on germ cell morphology. Therefore, the somatic expression of *sov*, at least from the time of clonal induction (first instar larvae), is sufficient to allow female germline development.

The requirement for *sov* activity in ovarian development is controlled by the somatic sex regulatory genes: The sex specificity of *sov* function for both the development of the somatic ovary and the completion of oogenesis indicates that *sov* is ultimately responding to the *X*:A ratio, the initial signal of sex determination. This can occur in one of two ways. Most aspects of the sexually dimorphic somatic differentiation of the gonad are regulated by the activities of the *transformer* (*tra*), *transformer-2* (*tra-2*) and *doublesex* (*dsx*) genes (BAKER and RIDGE 1980; BELOTE and BAKER 1982; WIESCHAUS and NÖTHIGER 1982). Although clonal analysis has demonstrated that *tra*, *tra-2* and *dsx* activity are not required in the female germline for oogenesis (MARSH and WIESCHAUS 1978; SCHÜPBACH 1982), these genes are similar to *sov* in that mutations in them cause necrosis and aberrant germ cell morphologies that are the indirect effect of disrupted female somatic development (MCKEOWN *et al.* 1988; STEINMANN-ZWICKY *et al.* 1989; STEINMANN-ZWICKY 1994). These observations could be explained by a mechanism where the somatic sex regulatory genes determine the state of *sov* activity, which in turn is required for the formation of the somatic ovary and the support of *X/X* germ cell development.

Alternatively, *sov* may repond to the the *X*:A ratio by a pathway independent of that controlled by *tra*, *tra-2* and *dsx*. At least one such pathway is known to exist for dosage compensation in males (BAKER and BELOTE 1983; LUCCHESI and MANNING 1987). The male-specific lethal genes (msls) control the hypertranscription of the male *X* chromosome but appear to have no role in females (BELOTE and LUCCHESI 1980; KURODA *et al.* 1991). The functions of these genes are not affected by mutations in *tra*, *tra-2* or *dsx*, indicating a separate mechanism for their sex-specific activity. The same may also be true for the regulation of *sov*.

One direct way to examine how sex-specific *sov* activity is regulated is by determining whether the loss of *sov* activity can alter the gonad mutant phenotypes associated with *tra*, *tra-2* or *dsx* mutations. Loss of function mutations in *tra* or *tra-2* and certain allele combinations of *dsx* result in chromosomally female (*X/X*) flies developing somatically as males (FUJIHARA *et al.* 1978; BAKER and RIDGE 1980). These *X/X* "pseudomales" produce malelike somatic testes (pseudotestes) that contain a degenerating and mophologically aberrant germ cell population (Figure 2A) (BROWN and KING 1961; BELOTE and BAKER 1982). If *sov* is regulated by the somatic sex regulatory genes to promote ovarian development, then *X/X* flies transformed to a male identity by mutations in *tra*, *tra-2* or *dsx* will not express the *sov* ovarian activity even if the sov^+ allele is present. Therefore, the presence of *sov* mutations in these pseudomales should have no effect on the pseudotestis phenotype. Alternatively, if *sov* reponds to the the *X*:A ratio by a pathway independent of that controlled by *tra*, *tra-2* or *dsx*, then the pseudotestis will still require *sov* function because of its *X/X* genotype. In this case, we would expect that *sov* mutations will disrupt the development of the *X/X* pseudotestis in a manner analogous to *sov* mutant *X/X* ovaries.

Our results indicate that the *sov* product is required in somatic cells undergoing ovarian development regardless of their *X*:A ratio. Typically, sov^+ pseudotestes are short with most of the germ cells located apically and in an undifferentiated and degenerating state (Figure 2A). sov^- pseudotestes have a range of gonadal phenotypes that are indistinguishable from those seen in sov^+ pseudomales (compare Figure 2A with B). This

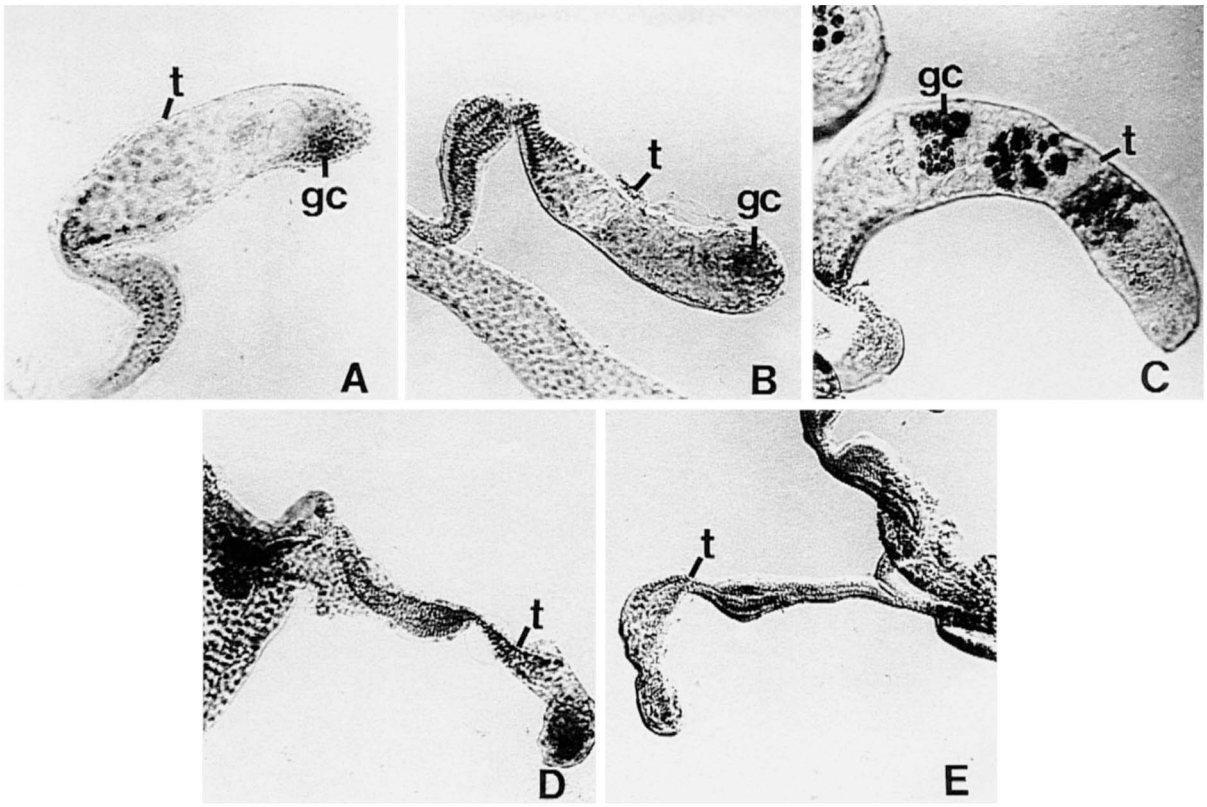

FIGURE 2.—The effect of *sov* mutations on the gonads of *X/X* pseudomales. (A) Pseudotestis from *X/X* flies transformed into a somatic male by a mutation in the *tra-2* gene. Germ cells (gc) proliferate into the adult stage but are largely undifferentiated. These flies have two copies of sov^+. (B) sov^2/sov^2, $tra\text{-}2^-$ pseudotestes are indistinguishable from sov^+/sov^+ pseudotestes. (C) sov^2/sov^+ pseudotestis from sibling of fly shown in B. Flies of this genotype typically give rise to large testes with multiple clusters of germ cells distributed throughout the lumen of the gonad. (D) An extreme mutant phenotype often seen in sov^2/sov^2, pseudotestes are gonads that are largely devoid of germ cells. Even in this extreme case, the somatic gonad still develops. (E) *X/Y* testis from a $tudor^-$ mother. The *tudor* mutation acts maternally to block germ cell development in both male and female progeny. Even in the absence of germ cells, the somatic gonad can still develop. The nuclei of the preparations were stained by Feulgen reaction. t, testis; gc, germ cells.

is true even with the most severely affected pseudotestes. In the absence of *sov* activity, many of the pseudotestes contain few germ cells, resulting in gonads that appear as elongated collapsed tubes (Figure 2D). We compared the morphology of these pseudotestes with those resulting from males derived from $tudor^-$ mothers. Mutations in *tudor* cause females to give rise to progeny that completely lack germ cells (BOSWELL and MAHOWALD 1985). *X/Y* testes that are somatically normal but lack a germline are virtually indistinguishable from the severe sov^- *X/X* pseudotestes (Figure 2E). These results indicate that *X/X* mesodermal cells that give rise to the somatic gonad do not require *sov* activity when developing as testes.

Support for this conclusion comes from the complementary experiment in which *X/Y* flies were transformed into "pseudofemales" by the ectopic expression of the *tra* gene. A transgenic fly strain was obtained that carried a construct in which the female-specific *tra* cDNA was fused to the *hsp83* promoter (from the laboratory of Dr. P. SCHEDL, Princeton University). At 25°, *X/Y* flies carrying one copy of this construct developed somatically as females. The ovaries of these *X/Y* pseudofemales generally produce egg cysts that contain hundreds of small undifferentiated cells (MCKEOWN *et al.* 1988) (Figure 3A). This phenotype is very similar to the "ovarian tumor" cysts resulting from mutations in the *otu* gene. When *X/Y* pseudofemales were made sov^-, the resulting gonads were severely deformed in a manner similar to sov^- *X/X* ovaries (Figure 3B). They lacked ovarioles and often the oviducts ended in "nubs" (see Figure 1D). These observations demonstrate that the sex-specific requirement for *sov* in gonadal development is controlled by the somatic sex regulatory genes, *tra*, *tra-2* and *dsx*.

In addition to the abnormal development of the somatic ovary, *sov* mutations also cause morphological aberrations and necrosis in *X/X* germ cells. We were interested in determining whether *X/X* germ cells developing in a male soma still required *sov* function. This was tested by examining the morphology of the germ cells produced in pseudotestes carrying different com-

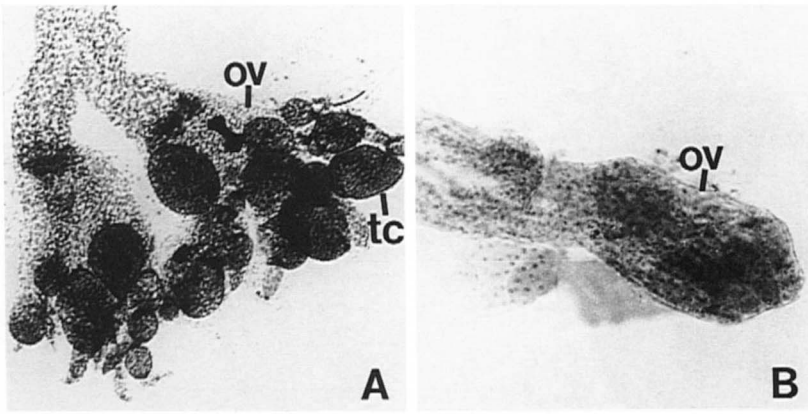

FIGURE 3.—The effect of *sov* mutations on the gonads of *X/Y* pseudofemales. (A) Pseudoovaries produced by the expression of the *tra* gene in an *X/Y* fly. The *tra* structural sequence was fused to the Drosophila *hsp83* heat shock promoter. At 25°, *X/Y* flies carrying a single copy of the *hsp-tra* construct are transformed to phenotypic females. The pseudoovaries contain egg cysts filled with hundreds of small, undifferentiated cells. (B) *X/Y* pseudoovary mutant for sov^2. These ovaries are small and rarely contain egg cysts, resembling severe sov^-, *X/X* ovaries. The nuclei of the preparations were stained by Feulgen reaction. ov, ovary; tc, tumorous cyst.

binations of wild-type and sov^- alleles. We classified the pseudotestes into three phenotypic categories. Group A is composed of agametic gonads in which few, if any, germ cells could be detected (Figure 2, D and E). Group B consists of gonads containing varying numbers of germ cells that appear either undifferentiated and degenerating or are arrested during early stages of spermatogenesis (Figure 2, A and B). Group C is represented by gonads carrying one or more polyploid cells that are morphologically similar to nurse cells (Figure 2C). These cells are often found in clusters separate from the less-differentiated germ cells and may represent an abortive attempt at oogenesis. We compared the pseudotestes phenotype of sov^-/sov^- pseudomales with their sibling sov^-/sov^+ pseudomale gonads. Both genotypes were derived from the same parents and were grown simultaneously under identical culture conditions. Both in turn were compared with sov^+/sov^+ pseudomales obtained in a separate set of crosses. Our results showed no consistent effect of different doses of

TABLE 4

The effect of *sov* dosage on the pseudotestes phenotype

Genotype	Group A (agametic)	Group B (male or undifferentiated)	Group C (nurse-like cells)	Total
$\frac{+}{+}; \frac{tra}{tra^{v1}}$	0.07	0.89	0.04	82
$\frac{sov^2}{+}; \frac{tra}{tra^{v1}}$	0.05	0.92	0.03	157
$\frac{sov^2}{sov^2}; \frac{tra}{tra^{v1}}$	0.03	0.80	0.17	113
$\frac{+}{+}; \frac{tra\text{-}2}{tra\text{-}2B}$	0.01	0.99	0	72
$\frac{sov^2}{+}; \frac{tra\text{-}2}{tra\text{-}2B}$	0.01	0.59	0.40	154
$\frac{sov^2}{sov^2}; \frac{tra\text{-}2}{tra\text{-}2B}$	0.24	0.67	0.09	70
$\frac{+}{+}; \frac{dsx^T}{dsx^1}$	0	0.78	0.22	54
$\frac{sov^2}{+}; \frac{dsx^T}{dsx^1}$	0	0.80	0.20	50
$\frac{sov^2}{sov^2}; \frac{dsx^T}{dsx^1}$	0.10	0.57	0.33	40

Results are given as ratio (observed pseudotestes:total) Genotypes: sov^2 = *y cv* sov^2 *v f.* tra^{v1} = kar^2 ry^5 tra^{v1} *red.* + = *FM6.tra-2B* = cn^2 *tra-2B bw.*dsx^1 = dsx^1 p^p.

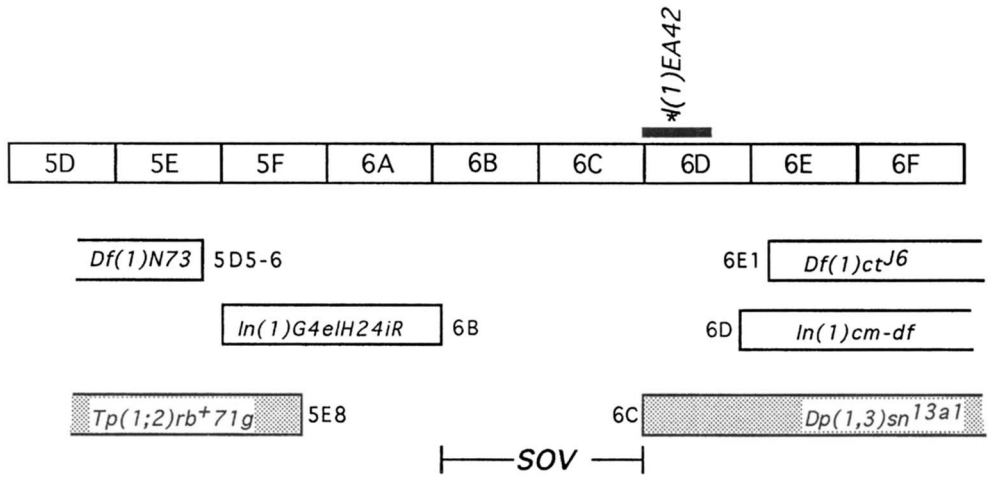

FIGURE 4.—Location of the *sov* locus on the cytological map of the *Drosophila melanogaster X* chromosome. A diagram of the banding pattern of the 6C-E region is shown with the approximate cytological location *l(1)EA42* (LEFEVRE 1981; LEFEVRE and WATKINS 1986). Sequences contained in relevant duplications are shown by a shaded box and regions deleted by deficiencies are denoted by an open box.

sov activity on the viability or differentiation of the *X/X* germ cells developing in pseudotestes (Table 4). Although there was substantial variability in the distribution of the phenotypic classes, no convincing pattern emerged in pseudomales resulting from mutations in *tra*, *tra-2* or *dsx*. Therefore, although *X/X* germ cells require somatic *sov* activity when developing in ovaries, this is not the case when developing in a somatic testis. This indicates that the male soma can support the viability of the *X/X* germline independent of *sov* function.

A curious result was seen in *tra-2* mutant pseudotestes heterozygous for sov^2 (Figure 2C; Table 4). Forty percent of the gonads produced by this genotype contained clusters of nurselike cells and similar high frequencies of this phenotype were consistently attained in multiple experiments with other *tra-2* allele combinations (data not shown). In contrast, nurselike cells were only rarely found in sibling sov^-/sov^- *tra-2* mutant pseudomales or in sov^+/sov^+ control pseudomales (Table 4). *dsx*-derived pseudomales also produced a substantial proportion of gonads (20–30%) that contained one or more clusters of nurselike cells, although in this case the phenotype was independent of the dosage of sov^+. The reason for these consistent shifts toward female germline differentiation is not clear but may reflect the effects of genetic background or the degree of male transformation caused by the alleles used.

The cytological and genetic mapping of the *sov* gene: Three female-sterile alleles of *sov*, $sov^{1,2,3}$, were isolated from a single EMS mutagenic screen (MOHLER 1977). Recombination mapping experiments localized the *sov* mutations to recombinant map position 18.5 on the *X* chromosome, placing it in the vicinity of cytological region 6C (MOHLER and CARROLL 1984). The *sov* mutations can be complemented by the deletions *Df(1)N73*, $Df(1)ct^{J6}$, *In(1)cm-df* and *In(1)G4eLH24iR* (Figure 4). In addition, two duplications were tested for their ability to rescue *sov*. Neither a duplication of 3F3–5E8 ($Tp(1;2)rb^+71g$) nor a duplication of 6D-7C ($Dp(1;3)sn^{13a1}$) included the *sov* gene. This places *sov* in the 6B-6C region (Figure 4).

Our initial studies suggested that the *sov* female-sterile mutations were allelic to a lethal mutation that mapped in the cytological interval between 6D1 and 6D7. We tested *sov* alleles against an EMS-induced lethal allele of *l(1)6Dd, l(1)EA42*. The lethal mutation was viable in all combinations with the *sov* alleles but resulted in female sterility and the formation of rudimentary ovaries. This inability to complement *sov* mutations indicates that the *l(1)EA42* chromosome is mutant for *sov* function. However, we found that the *l(1)EA42* lethality is separable from the *sov* mutant phenotype. The $Dp(1;3)sn^{13a1}$ duplication does not carry the *sov* gene, as shown by its inability to suppress the mutant phenotype of female-sterile *sov* alleles. However, this duplication can rescue the *l(1)EA42* lethality, although the surviving females are sterile and display an ovarian phenotype similar to severe *sov* mutations (Table 1). These data suggest that the *sov* and *l(1)EA42* mutations may map to separate genes.

The *sov* gene is associated with recessive lethality: A mutagenic screen was performed that was designed to isolate both sterile and lethal mutations on the *X* chromosome. Some 756 recessive lethals and 66 female-sterile EMS-induced mutations were individually tested for allelism with *sov*. The lethals were identified by the absence of hemizygous males. It was not possible to test the homozygous condition to confirm that they are also lethal in females. Two of the lethal lines gave rise to sterile females when made heterozygous with sov^2 and

are designated sov^{ML150} and sov^{ML185}. We believe that the lethal phenotype associated with sov^{ML150} and sov^{ML185} is due to the disruption of *sov* function. Recombination mapping of the lethality of both sov^{ML150} and sov^{ML185} place the mutations to within five map units of the *sov* gene (data not shown) and neither lethal allele are rescued by nearby duplications that do not contain the *sov* locus (*Tp(1;2)rb*$^{+}$*71g* and *Dp(1;3)sn*^{13a1}). In complementation tests with *l(1)EA42*, both sov^{ML150} and sov^{ML185} complement the nonsex-specific lethality of *l(1)EA42* but not the female sterility. Females that are sov^{ML150}/*l(1)EA42* and sov^{ML185}/*l(1)EA42* are fully viable, but most fail to produce ovaries (>95%, Table 1). The complementation between *l(1)EA42* and lethal alleles of *sov* support the contention that *l(1)EA42* is closely linked but not associated with the *sov* gene. Therefore, we tentatively designate the *sov* mutation on the *l(1)EA42* chromosome *sov4*.

When the *sov* female-sterile alleles were made heterozygous for sov^{ML150} or sov^{ML185}, the mutant ovarian phenotype became more severe. In sov^{2} homozygotes, >10% of the females completely lack one or both ovarian lobes and ~50% of the ovaries had a disorganized structure in which ovarioles were not detected (Table 1). When sov^{2} was made heterozygous with sov^{ML150} or sov^{ML185}, there was a substantial increase in the frequency of females absent one or both ovary lobes. The increase in the severity of the average mutant phenotype is consistent with sov^{ML150} and sov^{ML185} eliminating *sov* activity. From these results we propose that the *sov* lethal alleles represent null mutations in which *sov* activity is completely blocked, whereas the viable female-sterile alleles are hypomorphic lesions that either specifically disrupt an ovarian specific *sov* product or reduces *sov* function such that only ovarian development is affected.

DISCUSSION

***sov* is a somatic function required for both somatic and germline ovarian development:** The *sov* gene is essential for the development of the somatic ovary as well as the female germline. Mutations in *sov* result in a dramatic decrease in the size of the ovary, in the most severe cases causing the complete absence of the female gonad in the adult fly. In the intermediate phenotypes, the mutant ovaries display varying degrees of disorganization consistent with aberrant somatic development, including the absence of ovarioles, the formation of fused egg cysts and misshapen yolky egg chambers. The effects of *sov* mutations on the morphology and development of the germline are equally severe. *sov* mutant egg cysts generally contain abnormal numbers of nurse cells and rarely develop a recognizable oocyte. Many cysts carry pycnotic nuclei that likely represent instances of nurse cell degeneration. Less frequently, *sov* mutations can result in the formation of "tumorous cysts" similar to that seen in ovaries mutant for certain alleles of the *ovarian tumor* gene. These egg chambers contain hundreds of small undifferentiated germ cells that fill the entire egg chamber. These studies demonstrate that *sov* is required for the development of the somatic organization of the ovary and can also influence the viability and differentiation of the *X/X* germline.

Despite the severe effects of *sov* mutations on oogenesis, mosaic studies indicate that germ cells that are made sov^{-} during the embryonic and early larval stages can develop into functional oocytes. This can result from either one of two mechanisms. The first possibility is that the germline requires *sov* activity during the embryonic period. By the time the sov^{-} germline clones are made during the larval stages, the requirement for *sov* expression has passed. Alternatively, the *sov* gene may need to be expressed only in the somatic tissues, which in a cell nonautonomous manner exerts an essential function on germline development. We believe that the latter interpretation is more likely because the morphological examination of *sov* mutant ovaries indicate that relatively late stages in oogenesis are affected. For example, aberrations in the number and morphology of nurse cells and the increase in nurse cell mortality suggest that the *sov* mutations affect nurse cell/oocyte differentiation and viability, processes that occur late in larval development. This suggests that *sov* function is required well after embryogenesis and past the time of clonal induction.

***sov* is essential for organismal viability:** The deleterious effects of the female-sterile *sov* alleles appear to be completely sex specific. Examination of male fertility, fecundity and testis morphology failed to demonstrate any effect of these alleles on male gonadal development. However, we believe that *sov* is essential for the viability of the fly. In a mutagenic screen for *X*-linked lethals and steriles, the only two *sov* alleles isolated were associated with a recessive lethality. When the female-sterile *sov* alleles are made heterozygous with either lethal allele, the females are viable but their ovarian mutant phenotype becomes more severe than when homozygous. We therefore believe that the female-sterile alleles represent hypomorphic mutations in what is an essential gene for males and probably females as well. In this regard, *sov* appears similar to the neurogenic genes *Notch* and *Delta*. Null mutations in either of these loci result in embryonic lethality due to disruptions in neurogenesis. Hypomorphic alleles of either *Notch* or *Delta* can cause female sterility associated with defects in the differentiation of the somatically derived follicle cells. Because the focus of this study is on the role of *sov* on ovarian development, a detailed study of the lethal phenotype will be presented elsewhere.

The ovarian requirement for *sov* is regulated by the somatic sex differentiation genes: In flies with two *X*

chromosomes and two sets of autosomes, the *tra* gene is expressed and acts with *tra-2* to control the expression of the *dsx* gene, which in turn regulates the differentiation of female-specific somatic structures. This includes the development of the female gonad from certain mesodermal cells that surround the embryonic germline. In the absence of *tra* or *tra-2* function, or with certain combinations of *dsx* alleles, these same mesodermal cells take on a male identity and give rise to somatic testes. However, not all sexually dimorphic processes are controlled by the action of these somatic sex regulatory genes. For example, the twofold difference in *X*-linked gene expression between male and female flies, that is, dosage compensation, is regulated by a different genetic pathway involving male-specific lethal genes (reviewed in LUCCHESI and MANNING 1987). Similarly, germline sexual differentiation, the choice between spermatogenesis and oogenesis, requires germline-specific genes that act independent of *tra*, *tra-2* and *dsx* (reviewed in PARKHURST and MENEELY 1994). Further complexity is demonstrated by the finding that the sex-specific development of certain male abdominal muscles depends on the activity state of *tra* and *tra-2* but not *dsx* (TAYLOR 1992). These observations indicate that although each sex-specific process is ultimately controlled by the *X*:A ratio, they depend on different regulatory loci for the regulation of subsequent steps in sexual differentiation.

We were interested in determining the basis for the sex-specific requirement for *sov* activity in gonad development. Using flies sexually transformed because of mutations in the somatic sex regulatory pathway, we found that both *X/Y* and *X/X* mesodermal cells require *sov* activity for the development of ovaries but neither cell type requires *sov* for testis development. This indicates that the *sov* requirement in ovarian development can be separated from the *X*:A ratio by mutations in the somatic sex regulatory genes. Therefore, in the mesodermal precursors to the somatic gonads, the somatic sex regulatory genes *tra*, *tra-2* and *dsx* must act before *sov* to initiate sex-specific gonadal differentiation. The *sov* gene then responds to the sexual state of soma such that only cells with a female identity require the ovary-specific *sov* function. This sex- and ovary-specific regulation contrasts with the male, and presumably female, lethality associated with the absence of *sov* activity. Therefore, it appears that the *sov* viability and ovarian functions are controlled by different genetic pathways.

X/X germ cells do not require *sov* activity if developing in a male soma: Our findings also provide some insight into the sex-specific interactions between the soma and germline that affect the viability and differentiation of the germ cells. In *X/X* flies, *sov* mutations result in aberrant differentiation of the female germline and an increase in germ cell necrosis. Because *sov* function is soma dependent, this effect on the germline must reflect some undefined interaction between the somatic cells and germline that is essential for oogenesis. In the absence of sufficient *sov* activity in the soma, this interaction is disrupted and germline development is arrested. However, we find that if the *X/X* soma is transformed to a male differentiated state, then *X/X* germ cell proliferation can occur and is not affected by reductions in *sov* activity. This suggests that the pseudotestes soma can support *X/X* germ cell viability and proliferation by a mechanism different than that which occurs in a female differentiated somatic gonad. A similar effect occurs in the reciprocal experiment. When *X/Y* flies are transformed to a somatic female differentiated state, the pseudoovaries are capable of supporting substantial proliferation of the *X/Y* germ cells (Figure 3A). In the absence of *sov* activity however, the pseudoovaries contain few germ cells (Figure 3B). This indicates that ovarian somatic tissue, even if *X/Y*, cannot support the proliferation or viability of either *X/Y* or *X/X* germ cells in the absence of *sov* activity.

In conclusion, sex determination and differentiation in Drosophila are initiated by the interpretation of the *X*:A ratio and is subsequently controlled by a hierarchy of regulatory genes that have progressively greater specificity in their actions. The *Sxl* gene is required systemically for all aspects of sexually dimorphic characteristics, whereas the *tra*, *tra-2* and *dsx* genes are limited to controlling sexual differentiation in the somatic tissue. The sex-specific instructions imposed by *tra*, *tra-2* and *dsx* must in turn be read by a set of genes that have more defined roles in the differentiation of specific sexually dimorphic tissues. We believe that *sov* represents one of these genes. We propose that *sov* participates in the development of the somatic ovary in response to the sexually differentiated state of the gonadal precursors. This function of *sov* has apparently been appropriated for other developmental pathways as well because *sov* is also essential for both male and female viability. Both the ovarian and viability functions of the *sov* gene are under investigation.

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